Fabrication of Consistent MoS_2 Biosensors for Quantifying Cancer-Related Biomarker Molecules with Femtomolar-Level Detection Limit

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Atomically layered transition metal dichalcogenides (TMDCs) exhibit a significant potential to enable next-generation low-cost field-effect transistor (FET) biosensors that permit single-molecule-level quantification of illness-related biomolecules. Although individual MoS_2 FET biosensors have been fabricated and exhibited very high biodetection sensitivity,[1,2] the fabrication of multiple such devices with consistent biosensor responses capable of quantifying the biomolecule interactions has not been attempted.

In this work, we fabricated multiple sets of MoS_2-based transistor biosensors and demonstrated that these devices can be synergistically utilized to measure the concentrations of cancer-related biomarker molecules with very low abundance (*e.g.*, fM-level TNF-α cytokine samples) as well as quantify the affinity and kinetic properties of the biomarker-receptor pairs.

The MoS_2 FET biosensors were fabricated using our previously reported plasma-assisted nanoprinting approach.[3] As-fabricated MoS_2 FETs were integrated with microfluidic structures and functionalized with anti-human TNF-α antibody receptors for quantifying the kinetics of antibody-(TNF-α) binding (**Fig. 1**). In particular, the device structure illustrated in Fig. 1d was utilized to obtain equilibrium-state sensor responses with respect to TNF-α concentration (*i.e.*, to determine a standard curve). All our biosensors exhibited a TNF-α detection limit as low as 60fM despite the small molecular size of the cytokine biomarker (~ 17kDa) that renders its label-free detection at high sensitivity significantly challenging. Such a low detection limit was achieved in both linear and subthreshold regimes of the transfer characteristics of MoS_2 transistors (**Fig. 2** only displays the subthreshold-regime responses). In both transport regimes, the electrically measured sensor responses were calibrated into signal quantities independent of the transistor performance. All sets of transistor biosensors exhibited consistent relationships between calibrated sensor responses and TNF-α concentration. They generated a standard curve, from which the equilibrium constant of the antibody-(TNF-α) pair was extracted to be $K_D = 424 \pm 70$ fM. Based on this calibrated sensor model, the time-dependent association-dissociation kinetics of the antibody-(TNF-α) pair was further studied using the device structure illustrated in Fig. 1e and the association/dissociation rates of the antibody-(TNF-α) pair were measured to be $(5.03 \pm 0.16) \times 10^8$ $M^{-1}s^{-1}$ and $(1.97 \pm 0.08) \times 10^{-4}$ s^{-1}, respectively (**Fig. 3**).

This work laid an important foundation for leveraging the excellent electronic properties of emerging atomically layered semiconductors in bio-assay applications as well as advanced the critical research capability in analyzing the biomolecule interactions with fM-level detection sensitivities. Notably, such capability would enable selection of antibodies with a high binding constant with respect to a specific target biomarker molecule, thereby providing a means to further improve the selectivity and fidelity of immunoassay.

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[1] D. Sarkar, W. Liu, X. Xie, A.C. Anselmo, S. Mitragotri, and K. Banerjee, ACS Nano **8**, 3992-4003 (2014)
[2] Wang *et al.*, Small **10**, 1101-1105, (2014)
[3] H. Nam, S. Wi, H. Rokni, M. Chen, G. Priessnitz, W. Lu, and X. Liang, ACS Nano **7**, 5870-5881 (2013)

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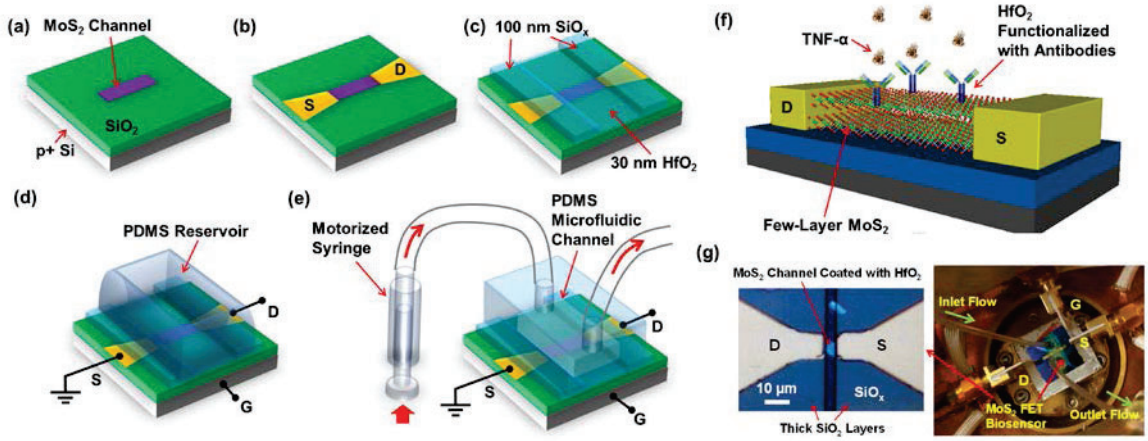

Figure 1. Flow chart for fabricating MoS_2 transistor biosensors: (a) printing of a few-layer MoS_2 flake onto a p^+-Si/SiO_2 substrate; (b) fabrication of Ti/Au D/S contacts; (c) ALD growth of the HfO_2 effective layer; (d) integration of a PDMS liquid reservoir on top of a MoS_2 transistor biosensor for obtaining equilibrium-state sensor responses with respect to TNF-α concentration; (e) integration of a microfluidic inlet/outlet tubing kit driven by a motorized syringe pump on top of a biosensor for real-time measurement of the kinetics of antibody-(TNF-α) binding/de-binding; (f) functionalization of the HfO_2 effective layer with antibody receptors and subsequent TNF-α detection. (g) An exemplary MoS_2 transistor biosensor and the measurement setup.

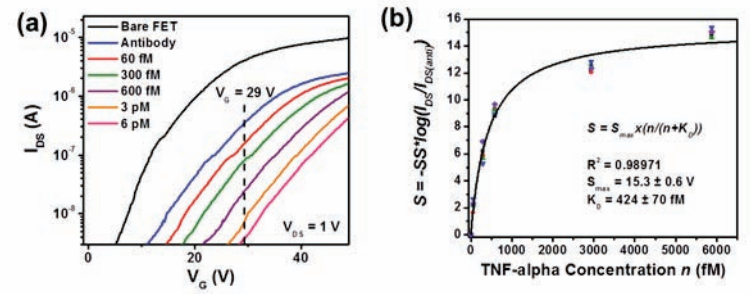

Figure 2. Sensor responses measured in the subthreshold regimes of MoS_2 transistor biosensors: (a) transfer characteristics of an exemplary MoS_2 transistor sensor measured at various biodetection stages, following the sequence of (1) bare transistor, (2) antibody functionalization, and inputs of TNF-α solutions with concentrations of (3) 60 fM, (4) 300 fM, (5) 600 fM, (6) 3 pM, and (7) 6 pM; (b) a set of calibrated subthreshold-regime responses (S) measured from five different MoS_2 transistor sensors with respect to TNF-α concentration (n).

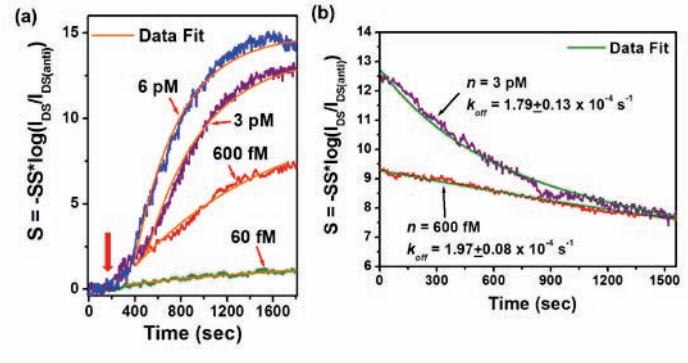

Figure 3. Time-dependent association and dissociation kinetics of the antibody-(TNF-α) pair: (a) real-time sensor responses of antibody-(TNF-α) binding measured under different TNF-α concentrations (n = 60 fM, 600 fM, 3 pM, and 6 pM); (b) Time-dependent dissociation kinetics of the antibody-(TNF-α) pair measured from n = 600 fM and 3 pM.

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